REMARKS

Claims 2-11, 15, 21, 23-26, 47-58 and 60-71 are active in this application. The claims are amended for clarity. Applicants have now submitted a Sequence Listing and a corresponding computer-readable Sequence Listing. Sequence Identifiers (SEQ ID NO:) have been added to the specification. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the Sequence Listing. Support for all of the sequences listed in the Sequence Listing is found in the present application as originally filed. No new matter is believed to have been introduced by the submission of the substitute Sequence Listing and the corresponding computer-readable Sequence Listing.

Applicants wish to thank Examiner Fredman for the courtesy of a discussion granted to the Applicants' undersigned representative by telephone on May 7, 2002. During this discussion, the undersigned pointed out that "TMR" is Bodipy TMR, to which the Examiner indicated a Declaration would be preferred to clarify this issue. To that end, Applicants provide herewith a Declaration from Mr. Kurata which avers to this fact. The undersigned also noted the claim amendments to Claims 2 and 47, i.e., selecting only specific fluorescent dyes and the advantages labeling the nucleic acid probes at the 5' end, as in Claim 60, and which allow further extension at its 3' end by a DNA polymerase, as in Claim 5. The Examiner indicated upon submission of the amendments and arguments, the rejections would be reconsidered. Therefore, Applicants respectfully request favorable reconsideration and allowance of all pending claims in light of the amendments submitted and remarks contained herein.

The present invention provides nucleic acid probes that solve the long-standing problem of those probes known previously (see page 4, lines 15-24 of the present specification) by enabling the determination of "the concentration of target nucleic acids in shorter times, more easily and more accurately" (page 2 , first paragraph of the present specification). The inventors have "found that emission of fluorescence from a fluorescent dye decreases (quenching phenomenon of fluorescence) when a nucleic acid probe labeled with the fluorescent dye hybridizes to a target nucleic acid. . . [the inventors have] also found that the extent of this decrease varies depending on bases in a probe portion, to which the fluorescent dyes is conjugated, or on the sequence of bases" (page 2, line 18 to page 3, line 1 of the present specification). Based on this discovery the present invention provides the presently claimed probes. The invention also provides kits and devices incorporating these probes and methods of determining the concentration of a nucleic acid.

The rejection of Claims 2, 3, 5, 6, 9, 46, 47 and 50 under 35 U.S.C. §102(b) over Horn et al (Nucleic Acid Research 1997 25(23):4842-4849) is obviated, in part, and respectfully traversed, in part.

With respect to Claims 2 and 47, these claims have been amended such that the fluorescent dye used to label the probe is not Bodipy FL.

Horn et al discloses various probes labeled at the 3' end with Bodipy FL (see page 4845, column 1, paragraph 3). Applicants note that Claim 5 recites that the nucleic acid probe is labeled at an end portion thereof and that the probe can be extended at its 3' end by a DNA polymerase. Thus, this nucleic acid probe can be used as a PCR primer, which Horn et al does not disclose(for example, see Figure 3 on page 4847 of Horn et al).

Therefore, the present claims are not anticipated by the disclosure of Horn et al. Withdrawal of this ground of rejection is requested.

The rejection of Claims 2-9, 23, 24, 47-50, 55, 56, 58, 60-63, 68, 69, 71 and 72 under 35 U.S.C. §103(a) over Horn et al (U.S. Patent No. 2001-0009760A1, Patent Publication) is respectfully traversed.

Horn et al discloses nucleic acid probes labeled with Bodipy FL at the 3' end of the probe (page 8). However, the Examiner has taken the position that it would have been obvious to put the label at the 5' end based on the Horn et al disclosure on page 10, column 1, paragraph 0087 (referring to pages 5 and 6 of the Official Action). Applicants disagree.

The nucleic acid probe as claimed in, for example, Claims 5 and 60 can be used as a PCR primer, which can be used to measure the time course of the reaction by decreased fluorescence in the absence of a quencher probe or a specific apparatus, such as, the evanescent wave detector as disclosed in U.S. Patent No. 5,750,337 (of record). In addition, that fluorescent intensity is significantly lower compared to a nucleic acid probe labeled with BODIPY FL at the 3' end because the fluorescent pigment of a 5' labeled probe is not released from the probe upon polymerase chain extension in the reaction, which release/degradation does occur when the probe is labeled at the 3' end. This release and degradation of the label at the 3' end is what is taught in Horn et al-- in column 10, line 1, paragraph 0087--"Taq polymerase will digest the probe from the 5' end and release a 5'-BODIPY®FL-nucleotide . . ." and is also depicted in Figure 3C.

The nucleic acid probes claimed in the present application provide a means of more accurately measuring the fluorescent emission from the probe because the background fluorescence associated with the release and degradation of the 3' end labeling is significantly

reduced. Therefore, the claimed probes provide a means to monitor PCR in real time. The fluorescently-labeled PCR products, containing the fluorescent labeled nucleic acid probes become fluorescent upon denaturation, which allows for the determination of melting temperature (Tm) based on the GC content and the nucleic acid probe length thereby providing a means to specifically analyze the melting curve from the Tm.

Therefore, the presently claimed nucleic acid probe provides a simple analytical tool for detecting, for example, specific polymorphisms in a gene as described in Example 23 of the present specification. Example 23 begins on page 107 and concludes on page 108, which states "it was ascertained from Figure 21, that the amplification products of the one copy and ten copies were different products as their TM values were different from each other."

Applicants again direct the Examiner's attention to the data provided in two Rule 132 Declarations demonstrating by Mr. Kurata provides data demonstrating that "all the probes labeled with the fluorescent dyes listed in the claim yield consisting quenching rates. . . " (see page 4 of the Kurata Declaration 2). However, the Examiner requested clarification as to whether the TMR label used in the second Kurata declaration is the same as BODIPY TMR and suggested that the claims would likely be allowable upon entry of the amendments submitted herein (Page 9 of the Official Action). Applicants confirm that, in fact, TMR is BODIPY TMR as evidence by the attached Declaration under 37 C.F.R. §1.132 executed by Mr. Kurata averring to that fact.

In view of the foregoing, the claims are not obvious in view of Horn et al and as such withdrawal of this ground of rejection is requested.

The rejection of Claims 2-11, 23, 24, 47-52, 55, 56, 58, 60-65, 68, 69, 71 and 72 under 35 U.S.C. §103(a) over the Horn et al prior art references in view of Metelev et al is respectfully traversed.

The core deficiencies of the Horn et al references are discussed at length above. In sum, neither Horn et al reference discloses the claimed nucleic acid probes labeled with specific dyes, as in Claims 2 and 47, nor the specific position of the label, as in Claims 5 and 60. Metelev et al only discloses using 2'-O-methyloligoribonucleotides in nucleic acid molecules but does not remedy any of the deficiencies of the Horn et al references, i.e., selecting the specific dyes as recited in, for example, Claims 2 and 47, or specific positioning of the dye as in Claims 5 and 60. Therefore, the present claims are not obvious in view of the Horn et al references combined with Metelev et al. Withdrawal of this rejection is requested.

The rejection of Claims 2-9, 15, 21, 23, 24, 47-50, 53-56, 58, 60-63, 66-69, 71 and 72 under 35 U.S.C. §103(a) over the Horn et al prior art references in view of Hogan et al is respectfully traversed.

The deficiencies of both Horn et al prior art references is discussed at length above. In sum, neither Horn et al reference discloses the claimed nucleic acid probes labeled with specific dyes, as in Claims 2 and 47, nor the specific position of the label, as in Claims 5 and 60. Hogan et al teaches using helper probes for enhancing hybridization. However, Hogan et al does not remedy any of the core deficiencies of the Horn et al prior art references, i.e., selecting the specific dyes as recited in, for example, Claims 2 and 47, or specific positioning of the dye as in Claims 5 and 60. Therefore, the present claims are not obvious in view of the Horn et al references combined with Hogan et al. Withdrawal of this ground of rejection is requested.

The rejection of Claims 2-9, 23-26, 47-50, 55-58, 60-63 and 68-72 under 35 U.S.C. §103(a) over the Horn et al prior art references in view of Heller et al is respectfully traversed.

The core deficiencies of the Horn et al prior art references are discussed at length above. In sum, neither Horn et al reference discloses the claimed nucleic acid probes labeled with specific dyes, as in Claims 2 and 47, nor the specific position of the label, as in Claims 5 and 60. Heller et al teaches stringency regulation by controlling temperature. However, Heller et al does not remedy any of the core deficiencies of the Horn et references, i.e., selecting the specific dyes as recited in, for example, Claims 2 and 47, or specific positioning of the dye as in Claims 5 and 60. Therefore, the present claims are not obvious in view of the combination of the Horn et al references and Heller et al. Withdrawal of this ground of rejection is requested.

The rejections of Claims 50-52, 56-58 and 64-65 under 35 U.S.C. §112, second paragraph, is obviated by amendment.

Applicants submit that the present application is now in a condition for allowance.

Early notice of such allowance is earnestly solicited.

Respectfully submitted,

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IN THE SPECIFICATION

Please amend the specification as follows:

Please replace the paragraph beginning on page 23, line 24, to page 24, line 22, with the following text:

--Incidentally, a nucleic acid probe with modified DNA, such as 2'-o-methyloligoribo-nucleotide, inserted in an oligodeoxynucleotide is used primarily for the determination of RNA. Upon determination of RNA by the probe, it is preferred to subject an RNA solution as a sample to heat treatment at 80 to 100°C, preferably 90 to 100°C, most preferably 93 to 97°C for 1 to 15 minutes, preferably 2 to 10 minutes, most preferably 3 to 7 minutes before hybridization with the probe such that the higher-order structure of RNA can be degraded. Where the base strand of the nucleic acid probe is formed of 35 or fewer bases, addition of a helper probe, for example, an oligonucleotide the base sequence of which is (5')AGGCCGGCCCTTGACTTTCCT(3') (SEQ ID NO. 1) to a reaction mixture is preferred for raising the efficiency of the hybridization to the sequence region. In this case, the helper probe can be in an oligodeoxyribonucleotide form or in a 2'-o-methyloligoribonucleotide form. When a nucleic acid probe longer than a 35-base strand is used, however, it is only necessary to thermally denature target RNA. When the nucleic acid probe according to the present invention is hybridized to RNA as described above, the fluorescence intensity decreases corresponding to the concentration of RNA in the reaction mixture, and RNA can be determined to a final RNA concentration of about 1.50 pM.--

Please replace the paragraph beginning on page 63, lines 9-14, with the following text:

--Preparation of a nucleic acid probe to be hybridized to the base sequence of a nucleic acid ranging from the 335th base to 358th base counted from the 5' end in 16S rRNA of *Escherichia coli*, namely, preparation of a nucleic acid probe having a base sequence of (3')CCGCTCACGCATC(5') (SEQ ID NO. 2) was conducted as will be described hereinafter.--

Please replace the paragraph beginning on page 63, line 16 to page 64, line 2, with the following text:

--A deoxyribooligonucleotide, which had the base sequence of (3')CCGCTCACGCATC(5') (SEQ ID NO. 2) and contained -$(CH_2)_7$-NH_2 bonded to the OH group on the carbon atom at the 3' position of deoxyribose at the 3' end of the deoxyribooligonucleotide, was purchased from Midland Certified Reagent Company, U.S.A. From Molecular Probes, Inc., "FluoroReporter Kit F-6082" (trade name) was also purchased, which contained not only "BODIPY FL" propionic acid succinimidyl ester but also a reagent for conjugating the compound to the amine derivative of the oligonucleotide. The kit was caused to act on the above-purchased oligonucleotide, whereby a nucleic acid probe labeled with "BODIPY FL" was synthesized for use in this Example.--

Please replace the paragraph beginning on page 66, line 15 to page 67, line 6, with the following text:

--An oligonucleotide, which was to be hybridized to 23S rNA of *Escherichia coli* JM109, had a base sequence of (5') CCCACATCGTTTTGTCTGGG (3') (SEQ ID NO. 3) contained -$(CH_2)_7$-NH_2, bonded to the OH group on the carbon atom at the 3' position of the

5' end nucleotide of the oligonucleotide, was purchased from Midland Certified Reagent Company, U.S.A. as in Example 1. From Molecular Probes, Inc., "FluoroReporter Kit F-6082" (trade name) was also purchased, which contained not only "BODIPY FL" propionic acid succinimidyl ester but also a reagent for conjugating the compound to the amine derivative of the oligonucleotide. The kit was caused to act on the above-purchased oligonucleotide, whereby a nucleic acid probe labeled with "BODIPY FL" was synthesized. The synthesized product so obtained was purified as in Example 1, whereby the nucleic acid probe labeled with "BODIPY FL" was obtained with a yield of 25% as calculated relative to 2 mM of the starting oligonucleotide.--

Please replace the text on page 69, line 1, with the following text:

Name Target deoxyribooligonucleotide (SEQ ID NOS. 4-13)

Please replace the text on page 69, line 12, with the following text:

Name Invention probe (SEQ ID NOS. 14-20)

Please replace the text on page 72, line 1, with the following text:

Name Target deoxyribooligonucleotide (SEQ ID. NOS. 21-31)

Please replace the text on page 72, line 14, with the following text:

Name Invention probe (SEQ ID NOS. 32-42)

Please replace the text on page 74, line 3, with the following text:

Name Target deoxyribooligonucleotide (SEQ ID. NOS. 43-46)

Please replace the text on page 74, line 9, with the following text:

Name Invention probe (SEQ ID NOS. 47-50)

Please replace the paragraph beginning on page 77, line 8 to page 78, line 13 with the following text:

--An oligonucleotide was purchased from Midland Certified Reagent Company, U.S.A. as in Example 1. The oligonucleotide had a base sequence of (5')CATCCCCACCTTCCTCCCAGTTGACCCCGGCAGTC(3') (SEQ ID. NO. 51) (35 base pairs) hybridizable specifically to the 16S rNA base sequence of KYM-7 strain, said base sequence being equivalent to the base sequence ranging from the 1156th base to the 1190th base of the 16S rRNA of *Escherichia coli* JM109, contained deoxyribonucleotides at the 1st to 16th bases and the 25th to 35th bases, respectively, said methyl-modified ribonucleotides being modified with methyl groups at the OH group at the 2' position on the carbon atom or ribose, and was modified with $-(CH_2)_7-NH_2-$ at the phosphate group of the 5'-terminal group of the 35 base pairs. From Molecular Probes, Inc., "FluoroReporter Kit F-6082" (trade name) was also purchased, which contained not only "BODIPY FLC6" propionic acid succinimidyl ester but also a reagent for conjugating the compound to the amine derivative of the oligonucleotide. The kit was caused to act on the above oligonucleotide, whereby a nucleic acid probe labeled with "BODIPY FL/C6" was synthesized. The synthesized product so obtained was purified as in Example 1, whereby the nucleic acid probe labeled with "BODIPY FL/C6" was obtained with a yield of 23% as calculated relative to 2 mM of the starting oligonucleotide. This probe was named "35-nucleotides chained 2-O-Me probe".--

Please replace the paragraph beginning on page 78, lines 14-21, with the following text:

--Using a DNA synthesizer, a riboxyoligonucleotide having a base sequence of (5')AGGCCGGCCCTTGACTTTCCT(3') (SEQ ID NO. 52) was synthesized as in the above to provide it as a forward-type hepter probe. On the other hand, a riboxyoligonucleotide having a base sequence of (5')AUGGGAGUUCAGUAGUACCCGCAAUGCUGGUCC(3') (SEQ ID NO. 53) was synthesized by using a DNA synthesizer, thereby providing it as a reverse type helper probe.--

Please replace the paragraph beginning on page 81, lines 12-15, with the following text:

--9) A ribooligonucleotide having a se sequence of (5')GUGACGGUCACUAUUUGACCUCCUUCCACCCC(3') (SEQ ID NO. 54) (35-base ribooligonucleotide).--

Please replace the paragraph beginning on page 81, lines 16-18, with the following text:

--10) A ribooligonucleotide having a base sequence of (5')GUGACGGUCACUAUUUG(3') (SEQ ID NO. 55) (17-base ribooligonucleotide).--

Please replace the text on page 83, line 11, with the following text:

(5') CATCCCCACCTTCCTCCGAGTTGACCCCGGCAGTC(3') (SEQ ID NO. 56)

Please replace the text on page 83, line 15, with the following text:

(5') CATCCCCACCTTCCTCTCGGCTTATCACCGGCAGTC (3') (SEQ ID NO. 57)

Please replace the text on page 86, lines 3-8, with the following text:

Invention probe: 3'TTTTTTTTGGGGGGGGC5' BODIPY FL/C6 (SEQ ID NO. 58)

Target nucleotide No. 1: 5'AAAAAAAACCCCCCCCA3' (SEQ ID NO. 59)

Target nucleotide No. 2: 5'AAAAAAAACCCCCCCCC3' (SEQ ID NO. 60)

Target nucleotide No. 3 : 5'AAAAAAAACCCCCCCCCI3' (SEQ ID NO. 61)

(I: hypoxanthine)

Target nucleotide No. 4: 5"AAAAAAAACCCCCCCCCI3' (SEQ ID NO. 62)

Please replace the paragraph beginning on page 87, line 17 to page 88, line 18, with the following text:

--A model of A DNA chip according to the present invention is illustrated in FIG. 6. Firstly, a modified probe and a surface-treated slide glass are provided first. The modified probe had been prepared by introducing an amino group onto the 3'-OH group at the 3' end of the invention probe, 3'TTTTTTTTGGGGGGGGC5' (SEQ ID NO. 63) BODIPY FL/C6, prepared in Example 13. On the other hand, the surface-treated slide glass had been prepared by treating a slide glass with a silane coupling agent which contained epoxy groups as reactive groups. A solution with the modified probe contained therein was applied in spots onto the surface-treated slide glass by a DNA chip production apparatus, "GMS™ 417 ARRAYER" (manufactured by TAKARA SHUZO CO., LTD., Kyoto, Japan). As a result, the modified probe is bound at the 3' end onto a surface of the slide glass. The slide glass is then placed for 4 hours or so in a closed vessel to bring the reaction to completion. The slide glass was alternately dipped in 0.1% SDS solution and water, twice in each of the solution and water, fox about 1 minute each time. Further, the slide glass was immersed for about 5 minutes in a boron solution, which had been prepared by dissolving $NaBH_4$ (1.0 g) in water (300 mL). Shortly after the slide glass was placed for 2 minutes in water of 95°C, the slide glass was alternately dipped in 0.1% SDS solution and water, twice in each of the solution

and water, for about 1 minute each time, so that reagents were washed off. The slide glass was then dried. As a result, a DNA chip according to the present invention was prepared.--

Please replace the paragraph beginning at page 89, line 20 to page 90, line 10, with the following text:

--A deoxyribooligonucleotide having a base sequence of (5')CATCGTTTACGGCGTGGAC(3') (SEQ ID NO. 64) was synthesized using a DNA synthesizer, "ABI394" (trade name: manufactured by Perkin Elmer, Corp.). An oligonucleotide, which had been prepared by treating the phosphate group at the 5' end of the oligodeoxyribonucleotide with phosphatase to form cytosine and then bonding $-(CH_2)_9-NH_2$ to the OH group on the carbon atom at the 5'-position of the cytosine, was purchased from Midland Certified Reagent Company. From Molecular Probes, Inc., "FluoroReporter. Kit F-6082" (trade name) was also purchased, which contained not only "BODIPY FL" propionic acid succinimidyl ester but also a reagent for conjugating the compound to the amine derivative of the oligonucleotide. The kit was caused to act on the above-purchased oligonucleotide, whereby Primer 1 of the present invention labeled with "BODIPY FL/C6" was synthesized.--

Please replace the paragraph beginning on page 91, lines 9-14, with the following text:

--Primer 2 composed of a deoxyribooligonucleotide, which had a base sequence of (5')CCAGCAGCCGCGGTAATAC(3') (SEQ ID NO. 65), and a fluorescent dye ("BODIPY FL/C6") labeled to the 5' end of the deoxyribooligonucleotide, was prepared with a yield of 50% in a similar manner as in Example 13.--

Please replace the text beginning on page 95, lines 4-6, with the following text:

- Forward primer E8F: (3')AGAGTTTGATCCTGGCTCAG(5') (SEQ ID NO. 66)

- Reverse primer E1492R: GGTTACCTTGTTACGACTT(5') (SEQ ID NO. 67)

c) Probe: BODIPY FL- (3') CCTTCCCACATCGTTT (5') (SEQ ID NO. 68)

Please replace the paragraph beginning on page 97, lines 1-17, with the following text:

--A deoxyribooligonucleotide having a base sequence of (5')CTGGTCTCCTTAAACCTGTCTTG(3') (SEQ ID NO. 69)was synthesized using a DNA synthesizer, "ABI394" (trade name; manufactured by Perkin Elmer, Corp.). An oligonucleotide, which had been prepared by treating the phosphate group at the 5' end of the oligodeoxyribonucleotide with phosphatase to form cytosine and then bonding $-(CH_2)_9-NH_2$ to the OH group on the carbon atom at the 5'-position of the cytosine, was purchased from Midland Certified Reagent Company. From Molecular Probes, Inc., "FluoroReporter Kit F-6082" (trade name) was also purchased, which contained not only "BODIPY FL" propionic acid succinimidyl ester but also a reagent for conjugating the compound to the amine derivative of the oligonucleotide. The kit was caused to act on the above-purchased oligonucleotide, whereby Primer KM38+C of the present invention labeled with "BODIPY FL/C6" was synthesized.--

Please replace the paragraph beginning on page 98, lines 16-18, with the following text:

--A deoxyribooligonucleotide having a base sequence of (5')GGTTGGCCAATCTACTCCCAGG(3') (SEQ ID NO. 70) was synthesized in a similar manner as in Example 18.--

Page 123 (Abstract), after the last line, beginning on a new page, please insert the attached Sequence Listing.

IN THE CLAIMS

Please amend the claims as follows:

Please cancel Claims 1, 12-14, 16-20, 22 and 27-45.

2. (Twice Amended) A nucleic acid probe for determining a concentration of a target nucleic acid, said probe being labeled with a fluorescent dye, wherein:

said probe is labeled at an end portion thereof with said fluorescent dye, and

said probe has a base sequence designed such that, when said probe is hybridized with said target nucleic acid, at least one G (guanine) base exists in a base sequence of said target nucleic acid at a position 1 to 3 bases apart from an end base portion where said probe and said target nucleic acid are hybridized with each other;

whereby said fluorescent dye is reduced in fluorescence emission when said probe is hybridized with said target nucleic acid, wherein said fluorescent dye is selected from the group consisting of [BODIPY FL, BODIPY FL/C3,] 6-joe, BODIPY TMR[, BODIPY FL/C6], Alexa 488, and Alexa 532.

24. (Twice Amended) The device according to claim 23, wherein said probes or said different [robes] probes are arranged and bound in an array pattern on said surface of said solid support.

47. (Twice Amended) A nucleic acid probe for determining a concentration of a target nucleic acid, said probe being labeled with a fluorescent dye, wherein:

said probe is labeled at an end portion thereof with said fluorescent dye, and

said probe has a base sequence designed such that, when said probe is hybridized with said target nucleic acid, base pairs in a probe-nucleic acid hybrid complex form at least one G (guanine) and C (cytosine) pair at said end portion;

whereby said fluorescent dye is reduced in fluorescence emission when said probe is hybridized with said target nucleic acid, wherein said probe has G or C as a 3' end base and is labeled at said 3' end thereof with said fluorescent dye, wherein said fluorescent dye is selected from the group consisting of [BODIPY FL, BODIPY FL/C3,] 6-joe, BODIPY TMR, [BODIPY FL/C6,] Alexa 488, and Alexa 532.

69. (Amended) The device according to claim 68, wherein said probes or said different [robes] probes are arranged and bound in an array pattern on said surface of said solid support.